EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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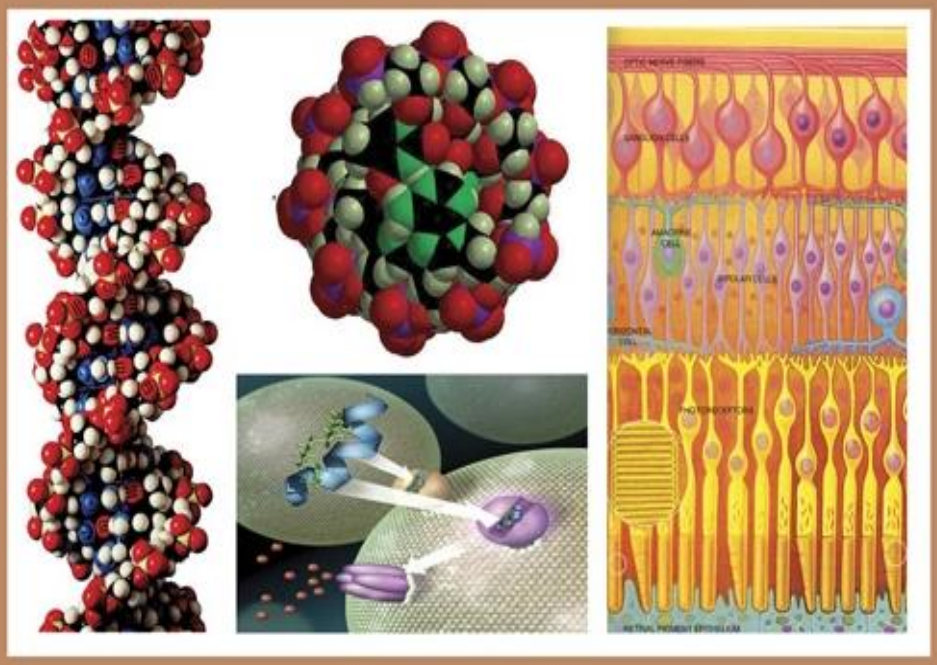


ISSN
2090-0767

WWW.EAJBS.EG.NET

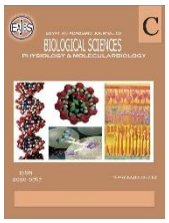
Vol. 14 No. 1 (2022)

.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(1) pp489-497 (2022)*
DOI: 10.21608/EAJBSC.2022.247431

Egypt. Acad. J. Biolog. Sci., 14(1):489-497 (2022)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Antioxidant and Anti-Inflammatory Activity of *Artemisia campestris L.*

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ARTICLE INFO
Article History
Received:1/5/2022
Accepted:25/6/2022
Available:27/6/2022

Keywords:
Artemisia campestris, total polyphenols, flavonoids, scavenger potential, anti-inflammatory activity.

ABSTRACT

This work focuses on a qualitative and quantitative study of some secondary metabolites of the species *Artemisia campestris* growing in the region of El Bayadh_Algeria. Accordingly, the total polyphenol content of the leaves is (89.12 ± 0.12 mg GAE / g) followed by stems and roots (85.11 ± 4.78 mg GAE / g) and (82.78 ± 4, 78 mg GAE / g) respectively. Moreover, the flavonoids content is also very high (119.12 ± 0.22 mg EC / g) for the leaves, (110.61 ± 0.09 mg EC / g) for the stems, and (105.71 ± 0.26 mg EC / g) for the roots. Therefore, the scavenger potential of free radicals of this species is very high. Henceforth, the classification of the three organs according to the DPPH test and the β-carotene bleaching test in descending order is leaves> stems> root. Thus, carrageenan in mice induced a remarkable anti-inflammatory dose-effect (600 mg/kg) recorded with an 82% inhibition of plantar edema.

INTRODUCTION

Mugwort (*Artemisia campestris* L) is a perennial plant belonging to the genus Artemisia of the Asteraceae family known as compositae (Varsha *et al.,* 2021)**.** It contains sturdy woody stems, striated at the base, 30 to 80 cm high (Ozenda, 1983), (Quezel P and Santa S, 1962), with dark green grained leaves (Figure 1), reddish twigs. This plant is locally named T'gouft (Megdiche *et al.,* 2015). It has tiny flower heads (1 to 1.5 mm) ovoid or conical, with scarious involucre. It contains only 3 to 8 yellowish flowers edged with red and a peduncle with whitish to brownish hairs. In addition, the fruits are achenes with a bitter flavor and a pleasant smell present in the semi-arid areas of the Mediterranean basin. This Mugwort is famous in the north, the highlands, and the Saharan Atlas, particularly in Hoggar (Algeria). It is widely used in traditional medicine to treat diabetes, rheumatism, scorpion stings, and snake bites (Al snafi *et al.,* 2015). Hence, according to Saoudi Met al. (2010) the daily intake of a decoction prepared from the leaves and stems of A. campestris helps reduce digestive symptoms. In this context, we chose to promote these traditional therapeutic uses. Thus, we evaluated the anti-radical and anti-inflammatory power of this species.

MATERIALS AND METHODS

Harvesting of Plant Material:

In October, we harvested the red Mugwort in the region of El Bayadh. After that, we cleaned the samples of the leaves, stems, and roots (Fig, 1).

Fig. 1. *Artemisia campestris,L*

Then, we put them to dry at room temperature in a ventilated (Moghtet *et al.*, 2020), (Singh *et al.*, 2011), shaded place to prevent sensitive molecules from heat and light. After grinding, using an electric grinder, we stored the vegetable powder in paper bags. We prepared the experiments in triplicate, where we expressed the results as the means with the standard deviation (Mamta *et al.*, 2011).

Preparation of Extracts:

The extraction was carried out by macerating (10g) of the plant powder from each organ (leaves, stems, and roots) for 24 hours with 100ml of a 70% hydro-ethanolic mixture. Then, we filtered the extract and stored it at 4 ° C. For the study of anti-inflammatory activity, we repeated the same protocol but with the whole aerial part, and we concentrated the filtrate to dryness with a rotavapor.

Screening Phytochemical:

We detected the chemical compounds by color reactions and different reagents: flavonoids (cyanidin reaction), alkaloids (reagent of Mayer), total phenols (folic-ciocalteu), reducing compounds (Fehling liquor), and coumarins with ammonia (Moghtet *et al.*, 2020), (Gheffour *et al.*, 2015).

Determination of Total Phenols:

The principle of the assay is the evaluation of the reducing power of ionic polymer compounds formed from the Folin-Ciocalteu reagent. Therefore, we introduced a volume of 200 µl of each extract into test tubes. Moreover, we added 1 ml of the Folin-Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate. We shook the test tubes and stored them for 30 minutes at room temperature. Absorbance was measured at 765 nm using a Jenway 6504 UV / VIS spectrophotometer (Boizot N and Charpentier JP *et al.*,2006**).** After that, we performed a calibration curve; in parallel under the same operating conditions using gallic acid as a positive control. We expressed the total phenol content of the plant extracts studied in milligrams (mg) gallic acid equivalent per gram of dry plant material (mg GAE / g) (Preeti *et al.*, 2013).

Determination of Flavonoids:

We evaluated the flavonoids by the oxidation of these phenolic compounds with aluminum trichloride (AlCl3)**.** Hence, we mixed 500 µl of each extract with 1500 µl of distilled water and 150 µl of 5% sodium nitrite (NaNO2). After standing for 5 min in the dark, we added 150 µl of 10% aluminum trichloride (AlCL3) to the mixture (Sarmistha *et al.*, 2014). After 11 min of incubation at room temperature, we added 500 µl of 4% sodium hydroxide (NaOH). We stirred the mixture to homogenize the contents. Subsequently, we read the absorbance of the pinkish-colored solution at 510 nm via a spectrophotometer. A calibration curve was carried out in parallel under the same operating conditions using catechin as a positive control (standard). We expressed the flavonoid content of the plant extracts studied in milligram (mg) equivalent

of catechin per gram of dry plant material (mg EC / g) (Dif *et al.*, 2015)

Evaluation of Antioxidant Activity:

DPPH (1,1-Diphenyl-2-picrylhydrazyl) Test (Ghanshyam *et al.*, 2014):

We added a volume of 50 µl of different concentrations of the methanolic extracts of the leaves and stems to 1.950 ml of the DPPH solution (0.025 g / l) freshly prepared with methanol. Also, we prepared the negative control in parallel by mixing 50 µl of the same solvent with 1,950 ml of DPPH. After incubation in the dark for 30 min at room temperature, the absorbance reading was taken at 515 nm using a spectrophotometer. After that, we calculated the percentage of radical trapping according to the following equation: % trapping = ((a1-a2) / a1) 100, where a1 is the absorbance of the control (solution of DPPH without extract), and a2 is the absorbance with it. We expressed the scavenger effect of the extracts vis-à-vis the DPPH radical by the 50% inhibitory concentration (IC50) that corresponds to the necessary one to inhibit or reduce 50% of the initial concentration of DPPH. A low IC50 represents the highest anti-free radical activity. We calculated All the IC50s graphically from the linear part of the inhibition curve percentages as a function of the concentration of the different extracts. The positive control is a standard solution of butyl hydroxyl-anisol (Gheffour *et al.*, 2015).

B-Carotene Test:

The test used is that of Guil-Guerrero JL *et al.* (2009) et Said M et al. (2020)**,** in which the presence of natural antioxidants such as total phenols and flavonoids reduces the destruction of β-carotene by neutralizing the hydroperoxides; which are formed by the oxidation of linoleic acid in the emulsion.

We dissolve 2 mg of β-carotene in 10 ml of chloroform, and we mix 1 ml of this solution with 200 mg of tween 40 and 20 µl of linoleic acid. After evaporation of the chloroform, we add 100ml of hydrogen peroxide. 200 µl of each extract prepared by the leaves, stems, roots, gallic acid, and catechin (2 mg/ml), are added to 5 ml of the emulsion then the tubes are incubated at 50 ° C in a water bath for 3 hours, and we measure the absorbance at 470nm. The negative control consists of 200 µl of methanol and 5 ml of the emulsion. We calculate the percentage of inhibition by the following formula (Cheurfa *et al.*, 2016): AA% = [1- (A0-At) / (A01-At1)] × 100, of which (A0 and A01) is Absorbance measured at zero incubation time of the extract and the control respectively, and (At and At1 :) is the absorbance measured after incubation.

Anti-inflammatory Activity:

We evaluated the anti-inflammatory effect of our extract by the test for inhibition of edema of mouse pulp induced by carrageenan (Ramesh *et al.*, 2010), (Kalkotwar *et al.*, 2013). This substance causes acute inflammation resulting in edema (Lakache *et al.*, 2021)**.** Randomly, we chose five groups of six mice, and they received the following solutions via intra-peritoneal way:

Batch n ° 1 (control): a solution of distilled water (10ml / kg).

Lot n ° 2 (with the reference molecule): diclofenac solution (10 mg/kg) (Manjula *et al.*, 2011).

Lot n ° 3: a solution of the extract (200 mg/kg).

Lot n ° 4: a solution of the extract (400 mg/kg).

Lot n ° 5: a solution of the extract (600 mg/kg).

One hour later (50 µl) of carrageenan (1%) was injected subcutaneously into the plant of the right hind leg. The thickness of each animal's leg was measured before treatment with carrageenan and afterward to monitor edema at 1, 2, 3, 4, 5, and 6 hours later. The percentage of inhibition (% PI) is calculated by the following formula**: % PI = (AB) / A × 100**, where A is the mean edema volume of the control group and B is the mean paw edema volume of the treated animal groups[14].

Statistical Analysis:

We expressed collected data in

mean ± standard deviation. As well, we performed statistical analysis using IBM SPSS, v 26. We realized the comparison between experimental groups using a one-way analysis of variance. Brown Forsyth and Welch tests followed if significant by Tukey or Games Howell post hoc tests. Differences are considered; important when $p \leq 0.05$, highly important when $p < 0.01$, and strongly important when $p < 0.001$.

RESULTS AND DISCUSSION

Phytochemical Screening:

The results of the colorimetric tests are presented in Table1. Alkaloids, reducing compounds, and coumarins are present but with moderately positive reactions for all three organs, while total phenols and flavonoids are present in huge quantities (strong presence) in the leaves, stems, and roots. Based on the results of this screening, quantitative analyzes were performed on the last two secondary metabolites.

Table 1. Phytochemical Screening

Plant's Part / Chemical Group	Leaves	Stems	Roots
Flavonoids	+++	+++	+++
Alkaloids	++	++	+
Total Phenols	+++	+++	+++
Reducing Compounds	++	++	++
Coumarins	++	++	++
Strong presence: +++; medium presence: ++; low presence +			

Determination of Total Phenols[23]:

The results of total phenol determination are shown in (Fig. 3). The extract of the leaves gave the highest content (89.12 ± 0.12 mg GAE / g) followed by the stems and roots (85, 11 ± 4.78 mg GAE / g) and (82.78 ± 4.78 mg GAE / g) respectively. These results are almost similarto those found by **(**15) on the same species in the region of Boussaada (88.61 ± 0.22 mg GAE / g) and Oum-El-Bouaghi (82.84 ± 0, 09 mg GAE / g) in Algeria. Another study by Bakchiche B A et al. (2019) recorded a higher content (102.09 ± 1.65 mg GAE / g) with a hydroalcoholic extract.

Determination of Flavonoids:

The concentrations of flavonoids in the three organs (leaves, stems, and roots) of red Mugwort are very high; (119.12 ± 0.22 mg EC / g), (110.61 ± 0.09 mg EC / g), (105.71 ± 0.26 mg EC / g) respectively (Fig. 5). These results are in agreement with the study of Ivana Ket al. **(**2011); where they found for the aerial part of the same plant and by varying the extraction methods the following contents: (102.5 ± 6.2 mg EC / g), (118.2 ± 3.0 mg EC / g), (104.5 ± 3.8 mg EC / g). On the other hand, Boudjouref M *et al.* (2018) found low concentrations by comparing them with our results (12.91 ± 0.01 mg EC / g) with the methanolic extract and 31.84 ± 0.00 mg EC / g) with an aqueous extract. These variations can be explained by the harvest period (the vegetative cycle, climatic conditions, and the nature of the biotope.

Antioxidant Activity (DPPH Test and β-carotene Test):

IC50 is inversely related to the antioxidant activity of a compound. Therefore, the lower the IC50 value, the greater the ability to scavenge free radicals. According to the results, it can be seen that the extract of the leaves has a great anti-free radical activity (IC50 = 0.23 ± 002 mg / ml) which is clearly superior to the reference antioxidant BHA (IC50 = 0.83 ± 03 mg / ml), followed by the extract of the stems (IC50 = 0.98 ± 12 mg / ml). On the other hand, the extract of the roots has a low capacity to trap free radicals (IC50 = 39.63 ± 0.006 mg/ml) compared to other organs. Our

results are identical to those obtained by the research of Akrout A *et al.* (2011); where they recorded an IC50 of 2.053 mg/ml from southern Tunisia and (15) that respectively found (IC50 = 0.241 ± 61.86 mg/ml) for a methanolic extract and (IC50 = 0.32 ± 22.58 mg/ml) for an aqueous extract in the region of Boussaada in the southeast of Algeria. The β-carotene bleaching test confirms the results of the first test in which the leaf and stem extracts showed a percentage of 92, 45 ± 0.23% and 90.47 ± 0.11%. These values are very close to the percentage inhibition of the reference substances (gallic acid: 95.18 ± 0.15% and catechin: 96.46 ± 0.38%), while the roots gave a relatively low percentage of 58.74 ± 0.35%. This biological activity is strongly promising in the field of pharmacology.

Anti-inflammatory Activity:

The carrageenan method has been used in this section for its simplicity and the rapid onset of edema after histamine release, serotonin, and leukocyte migration to the inflamed area (Boufadi *et al.,* 2016)**.** The results of EHa at different concentrations compared to the control batch (Table 2) induce highly significant prevention of the volume of plantar edema ($p < 0.001$) in mice from 1h and up to 6h. In particular, doses of 400 and 600 mg/kg, with a percentage of inhibition (Fig. 7) greater than diclofenac (82% at 2hours for EHa 600 mg/kg and 68.2 at 4hours for EHa 400 mg/kg) indicate better efficiency and highly relevant therapeutic interest. We confirmed this by the study of Zohra G. *et al.* (2016), which showed the effect of the aqueous extract of the same species on the significant decrease in the number of inflammatory cells.We also notice that the effect on the edema of the three solutions: diclofenac, EHa (200 mg/kg), EHa (400 mg/kg), and EHa (600 mg/kg); decreases at the 6hours, but it remains statistically highly significant and efficient when added to the control ($p < 0.001$), with inhibition indices 53.6; 51.3; 54.4; 47,1% respectively (Fig. 7) which suggests that our EHa inhibits the formation of inflammation mediators thanks to its richness in total phenols and flavonoids which are known for their anti-inflammatory activity.

Table 2. Evolution of the Plantar Edema Diameter over Six Hours in (mm)

Duration (Hours) / Extract(mg/kg)	1h	2h	3h	4h	5h	6h
Control	0,944± 0,071	0,915± 0,057	0,880± 0,069	0,932± 0,045	0,926± 0,034	0,933± 0,067
Diclofenac	0,339± 0,034***	0,385± 0,021***	0,298± 0,074***	0,308± 0,042***	0,363± 0,071***	0,433± 0,045***
***EHa* (200 mg/kg)**	0,750± 0,146	0,642± 0,043***	0,607± 0,054***	0,398± 0,062***	0,490± 0,067***	0,454± 0,049***
***EHa* (400 mg/kg)**	0,397± 0,049***	0,465± 0,060***	0,363± 0,060***	0,296± 0,052***	0,320± 0,067***	0,426± 0,094***
***EHa* (600 mg/kg)**	0,253± 0,046***	0,161± 0,062***	0,207± 0,055***	0,210± 0,082***	0,185± 0,087***	0,493± 0,102***

Statistically significant values compared to the control are indicated by the asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ **EHa**: Hydro-alcoholic extract

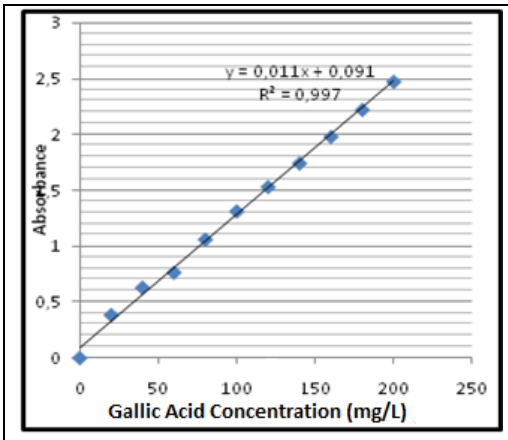

Fig.2.Standard curve of gallic acid.

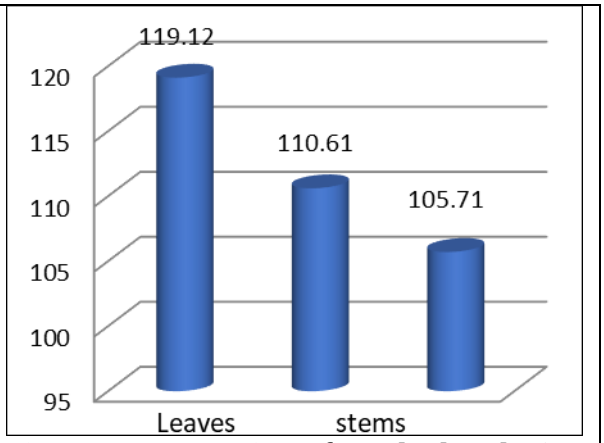

Fig. 3. Concentration of total phenols (mg GAE /g).

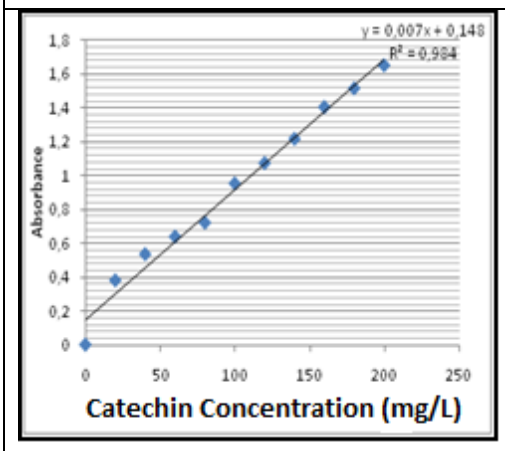

Fig. 4. Standard Curve of Catechin.

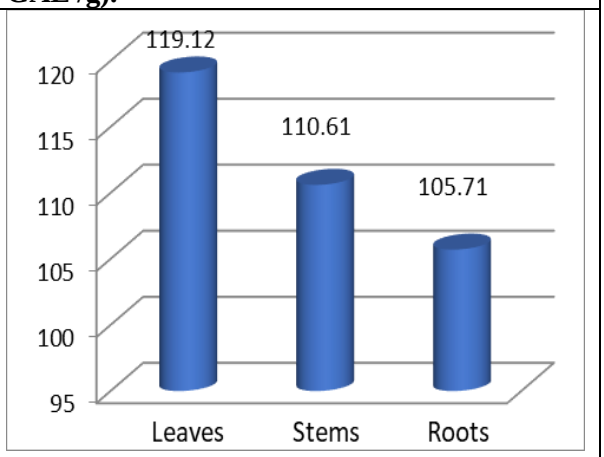

Fig.5.Concentration of flavonoids (mg CE /g).

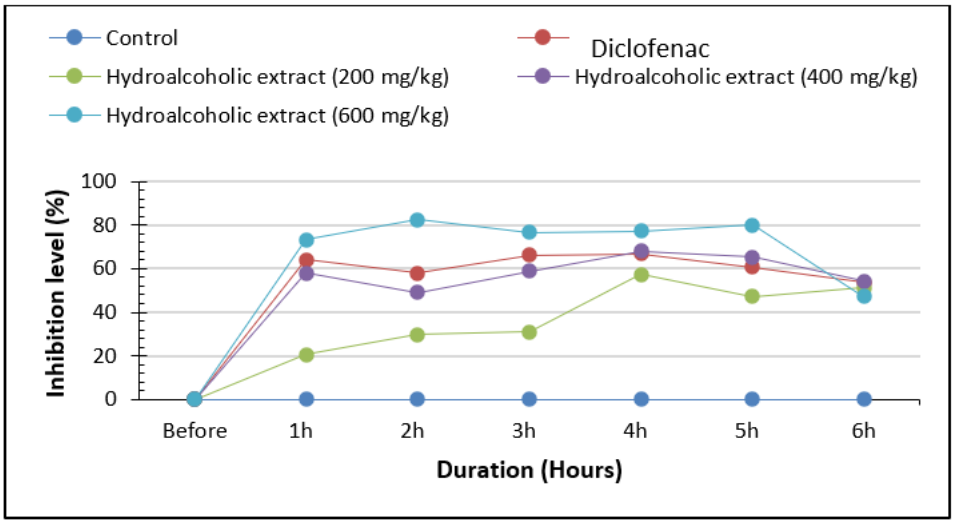

Fig. 6. Inhibition percentage of the inflammatory reaction in relation to time.

CONCLUSION

The result of our work shows that *Artemisia campestris*, L. is very rich in total phenols and flavonoids and that it has considerable antioxidant and anti-inflammatory activities, particularly in the leaves and stems, which justifies its wide use in traditional medicine. These data could disclose other studies with in-depth structural analyzes of the extracts for better use of this plant in pharmacology.

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